Genetic differences of exploratory and learning behavior reproduce reliably across labs and cohorts in mice housed under enriched conditions

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Board HH13

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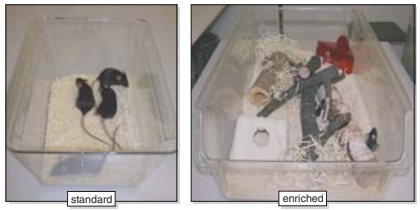
Summary

Standard animal housing conditions as typically used in biomedical research impose constraints on behavior that alter brain function and may question the validity of conclusions drawn from behavioral experiments. Enriched housing could solve this problem but is suspected to increase variability and to reduce the reliability of results. To address this question, we have run a multi-lab study involving 432 mice. We compared DBA/2 and C57BL/6 mice and their F1 cross in tests of exploratory activity and anxiety and in a water-maze place navigation task. Different teams used identical procedures in three labs (Anatomy Zurich, Psychiatric Research Zurich, Animal Welfare Giessen). Each lab tested three cohorts. In each, one half of mice was housed under standard conditions, the other in enriched cages:



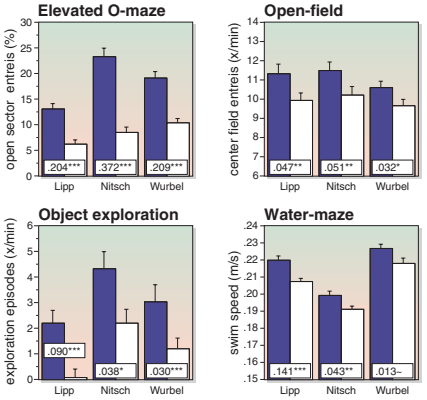
As expected, mouse strains showed marked performance differences in all tests. Strain rankings reproduced well across labs despite some variation of the magnitude of the differences. In all labs and independently of strain, enriched housing enhanced exploratory activity in the test arenas, but did not improve performance in the place navigation task. The rank order of strains was not affected by the housing conditions. On average, effects of strain and housing accounted for ca. 1/4 of the variance in the data, with effects and interactions of lab and cohort contributing another 1/4. Consistent detection of small strain differences was limited by within group variability rather than lab effects or interactions. Enrichment had no consistent effect on the partitioning of variance.

We conclude that the employed behavioral procedures allow reliable detection of genetic differences by different labs and teams under both standard and enriched housing conditions, provided that sample sizes are large enough and statistics interpreted carefully. The analysis of exploratory behavior is likely to benefit from enriched housing, in particular in strains with low base-line activity.

Procedure details:

Mice (C57BL/6, DBA/2, F1) housed under standard or enriched conditions as soon as obtained from supplier at weaning age. Each lab: 24 mice per strain and housing condition, in 3 cohorts. Begin of testing at 9 weeks. Elevated O-maze: diam. 46 cm, width 5.5 cm, height 40 cm, 1 session of 5 min starting in a closed sector. Open-field: arena 50x50 cm, walls 37 cm, 1 session of 5 min. Object exploration: arena as open-field, 15 min habituation, 15 min with unknown object in arena center (50 ml Falcon tube). Water-maze place navigation: pool diameter 150 cm, goal 14x14 cm hidden at constant position, water 24-26°, 4 days x 4 trials with 10 in ITI, max trial duration 90 s, 60 s probe trial on day 5. All experiments during active phase in dim light (4x40W, 12 lux), tacked using Noldus EthoVision 3.0 (www.noldus.com), data analysis in Wintrack 2.4 (www.dpwolfer.ch/wintrack).

1 Enrichment effects reproduce across 3 labs



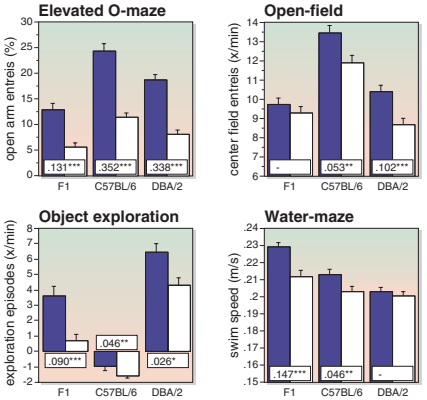
	enrich.	lab	enrich. x lab
O-maze	.269***	.094***	.039***
open-field	.047***	.009~	-
object expl.	.053***	.018**	-
water-maze	.054***	.268***	-

Selected effects/interactions from 4-way factorial ANOVA enrichment-strain-lab-cohort. Effects shown as partial omega squared, the proportion of variance accounted for by genotype if only this factor were in the design (range 0 to 1.0). *** p<.001, ** p<.01, * p<.05, ~ p<0.1. One representative variable shown for each test.

■ enriched, □ standard housing

Enrichment improved exploration on the O-maze, during the first 10 min in an open-field, and in an object exploration task. Only enriched mice showed significant object exploration in all 3 labs. Learning in the water-maze was not affected (not shown), but swim speed was increased. Despite varying baseline, enrichment effects were seen in all labs, some times with varying effect size.

2 Enrichment is beneficial for all 3 tested strains



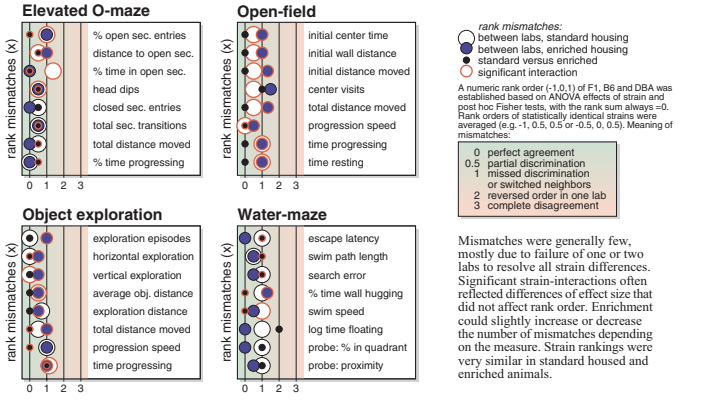
	enrich.	strain	enrich. x strain
O-maze	.269***	.153***	.004*
open-field	.047***	.426***	-
object expl.	.053***	.412***	-
water-maze	.054***	.164***	.017**

Selected effects/interactions from 4-way factorial ANOVA enrichment-strain-lab-cohort. Effects shown as partial omega squared, the proportion of variance accounted for by genotype if only this factor were in the design (range 0 to 1.0). *** p<.001, ** p<.01, * p<.05, ~ p<0.1. One representative variable shown for each test.

■ enriched, □ standard housing

Despite strong variation of baseline, enrichment effects had consistent direction across strains and procedures, but were in part too small to reach significance. In the object exploration task, standard housed C57BL76 mice showed object avoidance which was reduced but not corrected by enrichment. F1 mice showed significant object exploration only under enriched conditions.

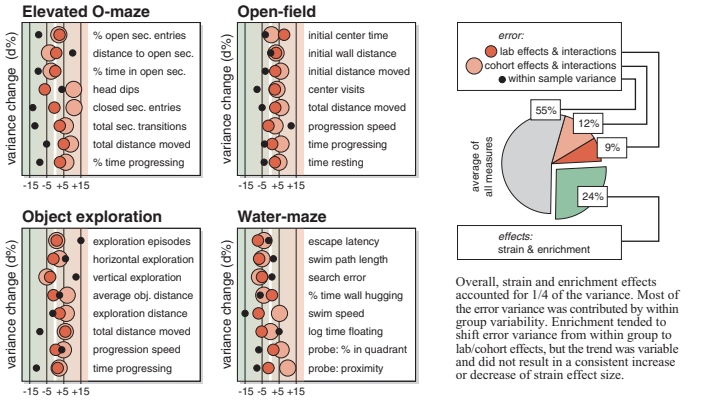
3 Environment has little effect on strain ranking



A numeric rank order (-1,0,1) of F1, B6 and DBA was established based on ANOVA effects of strain and post hoc Fisher tests, with the rank sum always =0. Rank orders of statistically identical strains were averaged (e.g. -1, 0.5, 0.5 or -0.5, 0, 0.5). Meaning of mismatches:

Mismatches were generally few, mostly due to failure of one or two labs to resolve all strain differences. Significant strain-interactions often reflected differences of effect size that did not affect rank order. Enrichment could slightly increase or decrease the number of mismatches depending on the measure. Strain rankings were very similar in standard housed and enriched animals.

4 Enrichment has no net effect on variability



Overall, strain and enrichment effects accounted for 1/4 of the variance. Most of the error variance was contributed by within group variability. Enrichment tended to shift error variance from within group to lab/cohort effects, but the trend was variable and did not result in a consistent increase or decrease of strain effect size.